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Effect of Intermittent Hyperoxia on Stem Cell Mobilization and Cytokine expression

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Persistent post concussion research

Table 2. Changes From Baseline in Postconcussion Symptom Scores Using the Rivermead Post-Concussion Symptoms Questionnaire and Subscales Among the Intent-to-Treat and Per-Protocol Populations[a]

Intervention	Intent-to-Treat Population				Per-Protocol Population		
	Baseline, Mean (SD)	After Intervention, Mean (SD)	Change Score (95% CI)	*P* Value[b]	Baseline, Mean (SD)	After Intervention, Mean (SD)	Change Score (95% CI)
Rivermead Post-Concussion Symptoms Questionnaire 3 Subscale							
Standard care	5.4 (2.7)	5.1 (2.8)	0.0 (−1.0 to 1.0) (n = 20)	.97	5.4 (2.7)	5.1 (2.8)	0.0 (−1.0 to 1.0) (n = 20)
HBO	5.5 (3.3)	4.2 (3.0)	1.2 (0.0-2.4) (n = 23)	.04	4.7 (3.2)	3.1 (2.2)	1.6 (−0.1 to 3.3) (n = 11)
Sham	4.7 (3.1)	3.5 (3.3)	1.5 (0.1 to 2.9) (n = 21)	.03	4.8 (3.7)	2.7 (2.8)	2.2 (0.7 to 3.6) (n = 13)
Rivermead Post-Concussion Symptoms Questionnaire 13 Subscale							
Standard care	27.1 (12.2)	25.5 (13.9)	0.5 (−4.0 to 5.0)	.87	27.1 (12.2)	25.5 (13.9)	0.5 (−4.0 to 5.0)
HBO	27.5 (13.1)	22.5 (12.4)	4.2 (−0.8 to 9.1)	.02	25.0 (13.4)	15.6 (10.9)	9.4 (2.9 to 15.9)
Sham	25.5 (11.6)	20.7 (12.8)	5.5 (0.7 to 10.3)	.04	25.9 (14.0)	17.4 (13.3)	8.5 (2.8 to 14.2)
Total Rivermead Post-Concussion Symptoms Questionnaire							
Standard care	32.5 (14.4)	30.6 (16.1)	0.5 (−4.8 to 5.8)	.91	32.5 (14.4)	30.6 (16.1)	0.5 (−4.8 to 5.8)
HBO	33.0 (15.8)	26.7 (14.8)	5.4 (−0.5 to 11.3)	.008	29.7 (16.3)	18.7 (13.0)	11.0 (3.2 to 18.8)
Sham	30.2 (14.2)	24.2 (15.4)	7.0 (1.0 to 12.9)	.02	30.8 (17.6)	20.1 (15.7)	10.7 (3.9 to 17.5)

Abbreviation: HBO, hyperbaric oxygen.

[a] The 95% CIs were calculated using 95% binomial exact CIs.

[b] Wilcoxon signed rank test.

1.5, 1.2 x40 n=71

Miller et al 2015

Conclusions

Conclusions

Among service members with PCS, HBO showed no benefits over an air sham compression procedure, but symptoms in both groups improved compared with mTBI care without supplemental chamber interventions.

This outcome suggests that the observed improvements were not oxygen mediated but may reflect nonspecific improvements related to placebo effects.

Taken with results from other concurrent investigations, our study does not support phase 3 trials of HBO for the treatment of PCS at this time.

Miller et al 2015

Experts would never consider room air (21% oxygen), delivered at 1.2 ATA, to be an acceptable therapeutic dose for any approved clinical indication.

It is illogical to think that this dose will have a biologically beneficial effect for any approved indication.

Hoge and Jonas, JAMA Internal Medicine 2015

Question

- Was this really a placebo effect or did both groups improve because of treatment with different doses of oxygen

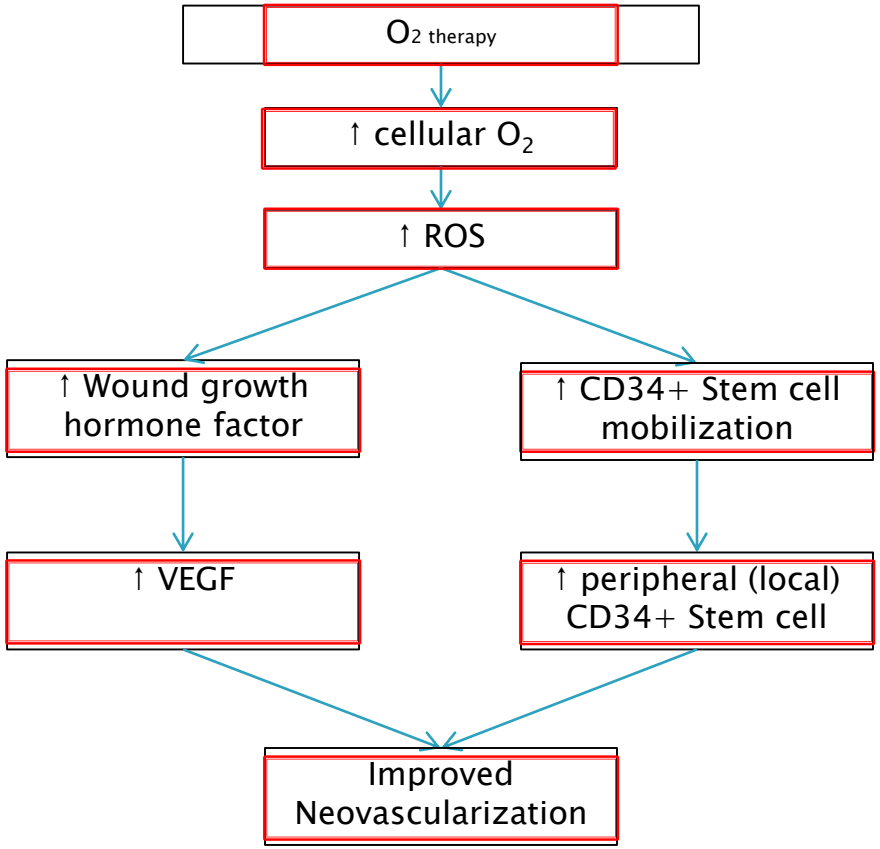
Acronym “CD”

- Cluster of differentiation
- Cell surface markers
- Identify stem cell sub-populations

- CD34
- CD133
- CD45

- “pro-angiogenic stem cells”

Pathway



Adapted from Thom, S.R *et al*. 2011

Stem cell mobilization by oxygen

- CD34+ stem cell in humans
- 1520 torr PiO_2
  - (2.0ATA x 100%O_2 x 760 torr)
- Suggesting a dose response
- Modulating SC mobilization

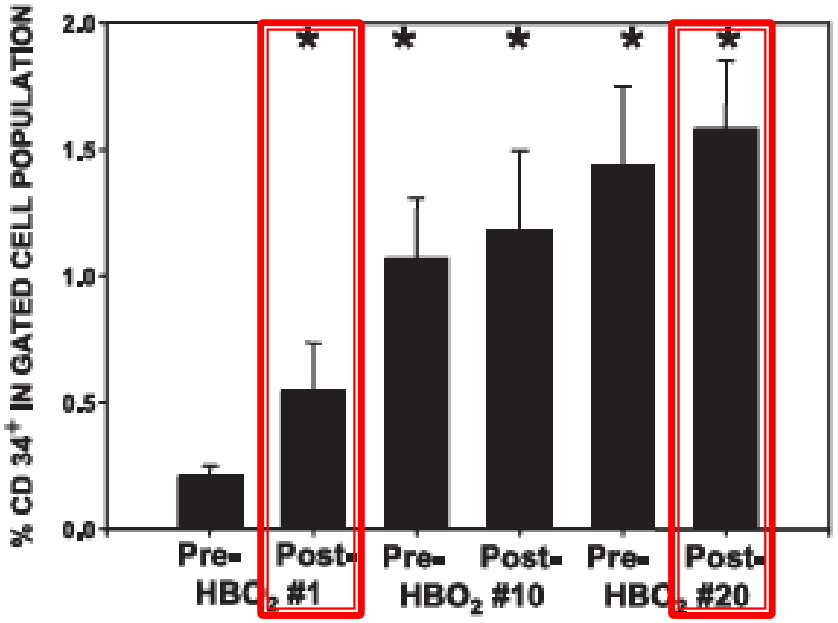


Fig. 4. Mean $CD34^+$ population in blood of humans before and after HBO_2 treatments. Data are the fraction of $CD34^+$ cells within the gated population using leukocytes obtained from 26 patients before and after their 1st, 10th, and 20th HBO_2 treatment. *Repeated-measures one-way ANOVA, $P < 0.05$ vs. the pre-HBO_2 first treatment value.

(Thom, Bhopale et al. 2006)

My?

Question

- Will similar physiologic activity (pro-angiogenic stem cell mobilization) be seen at $PiO_2 \leq 760$ Torr (100% O_2 @ 1ATA)

- 319 Torr PiO_2 (42% O_2)

Specific Aims

Specific Aim 1:

Determine the extent of proangiogenic stem cell mobilization in venous plasma in response to intermittent hyperoxia (↑PiO_2 319 torr) compared to control (room air PiO_2 ~159 torr).

Hypothesis:

Proangiogenic stem cells will be significantly mobilized

Specific Aims

Specific Aim 2:

Determine the expression of VEGF and TNFα in venous plasma after exposure to intermittent ↑PiO_2 319 torr.

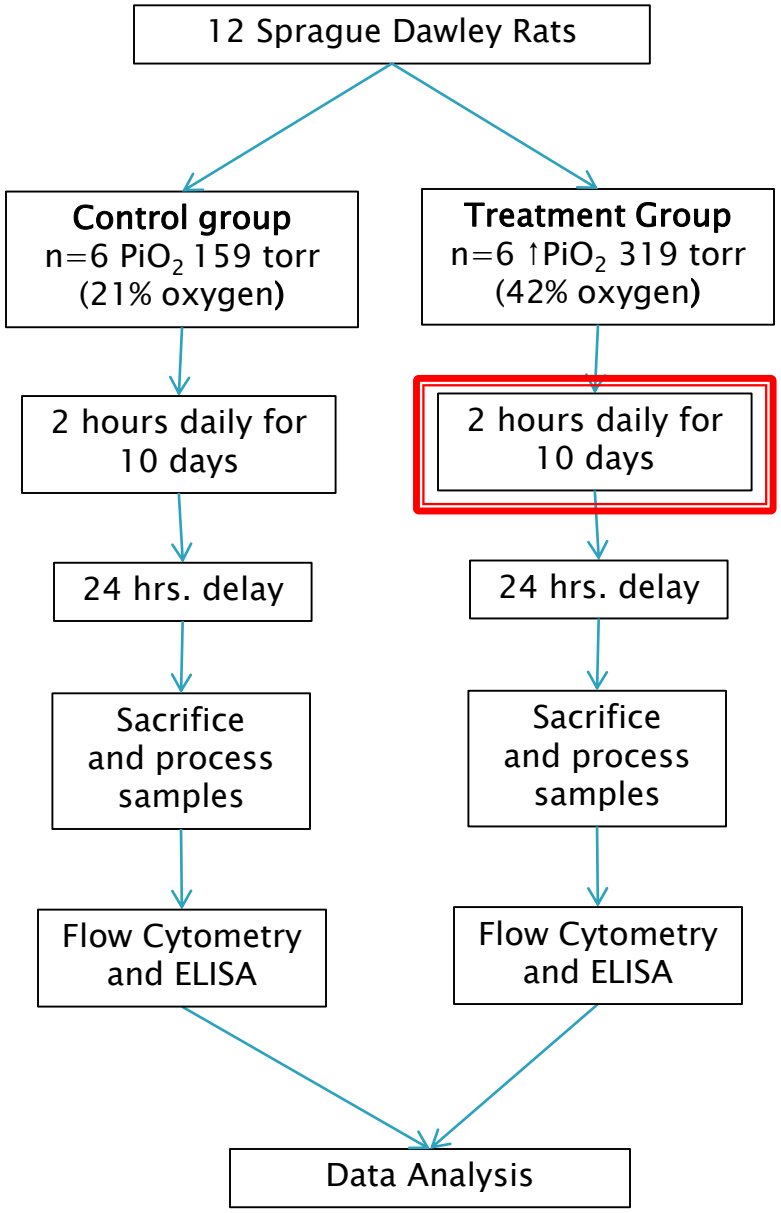
Hypothesis

TNFα expression will significantly decrease and VEGF will increase

Treatment setup 319 torr ↑PO_2

Treatment setup

Approach



Sample collection

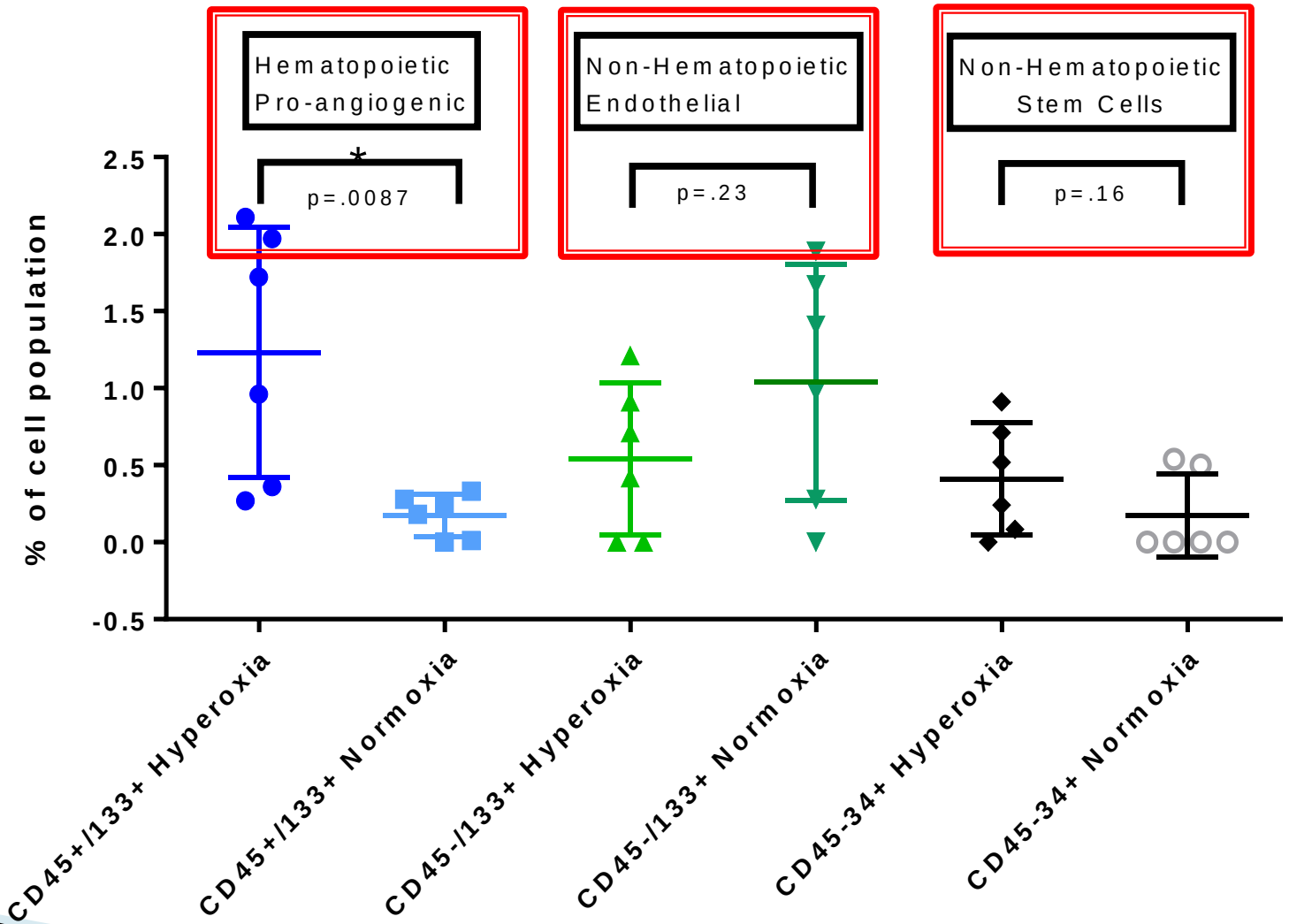
Methodology – Sample preparation

- Draw >8 mL venous blood from IVC into heparin tubes
- Lyse red blood cells – ammonium chloride
- Centrifuge to separate cells from plasma
- Freeze plasma for cytokine ELISA
- Stain cells with antibodies
- Quantify stem cells with flow cytometry
- Quantify cytokine expression with ELISA

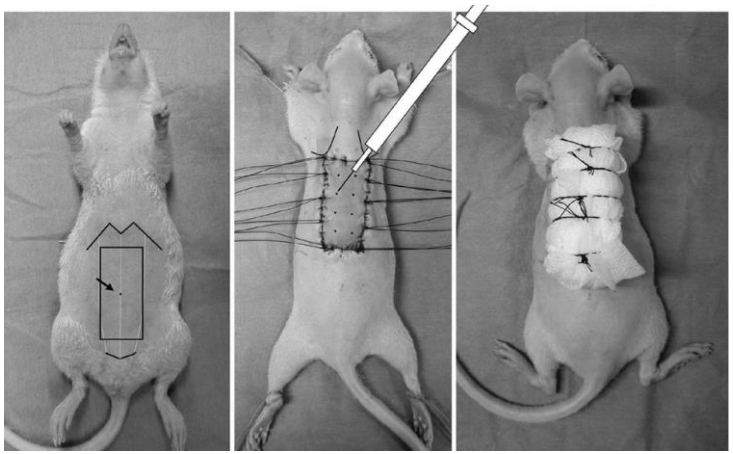
Data Analysis

- Non-parametric Mann Whitney test
- using a p of $< .05$ to indicate a difference between the groups

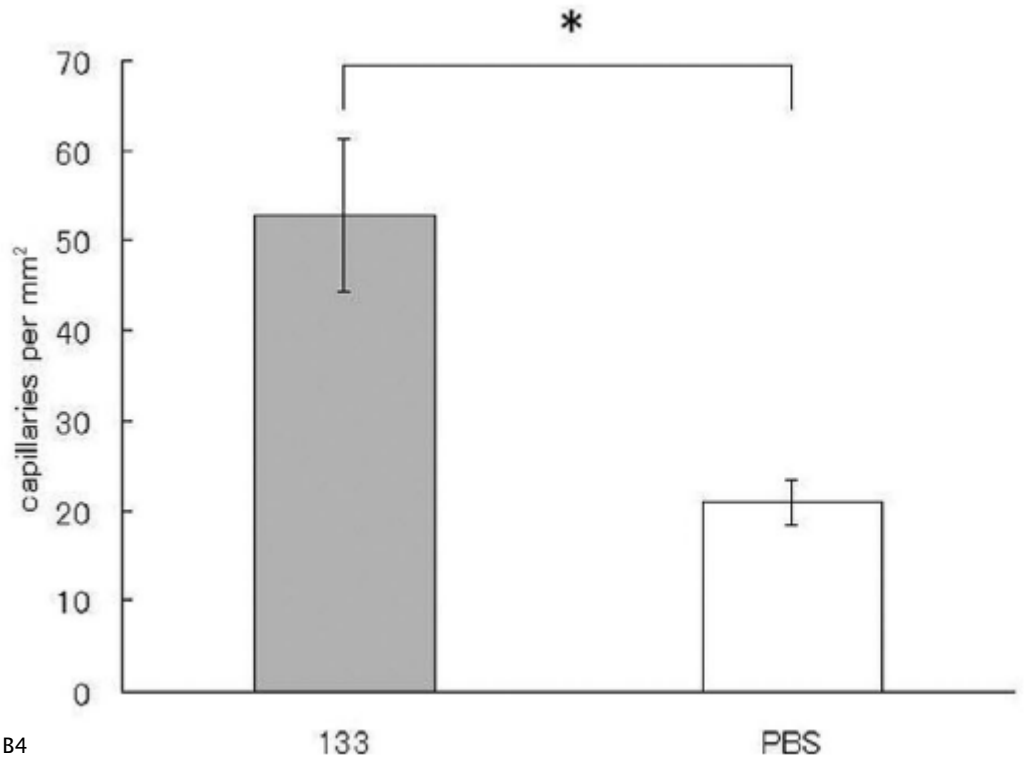
Results Pro-angiogenic stem cells



CD133+ neovascularization



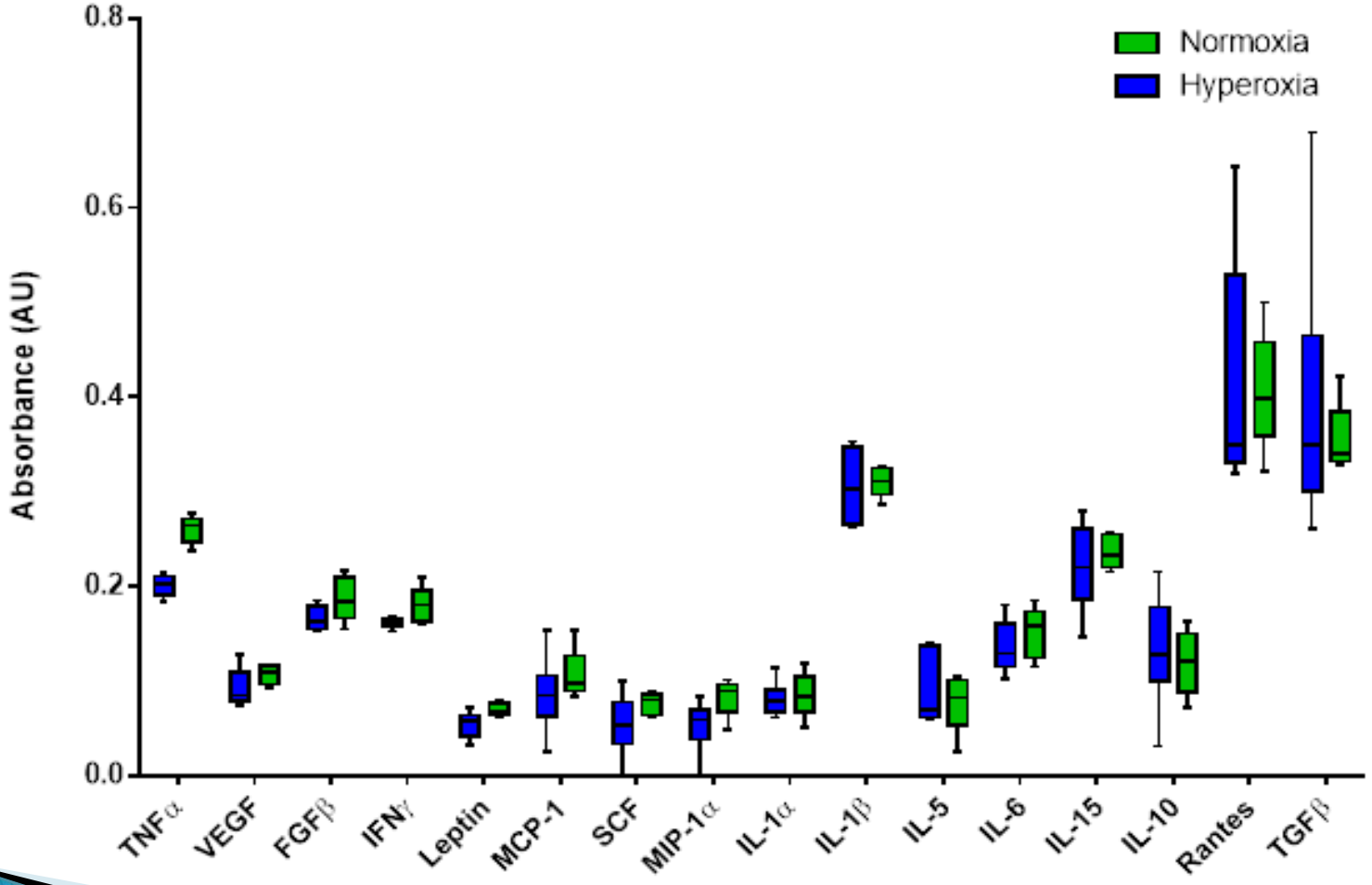
immunohistochemical staining using antibodies specific EC marker isolectin B4



Suggesting CD133 modulates vascular healing

(Nakanishi, Ishikawa et al. 2009)

Results – Cytokine Expression



TNF VEGF

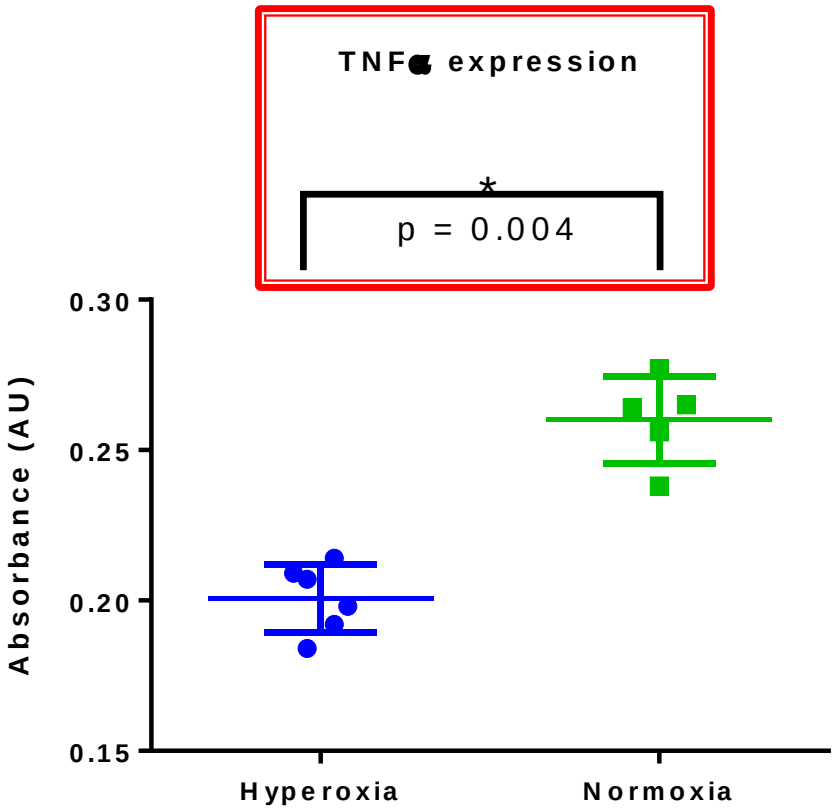
Tumor Necrosis Factor α (TNF α)

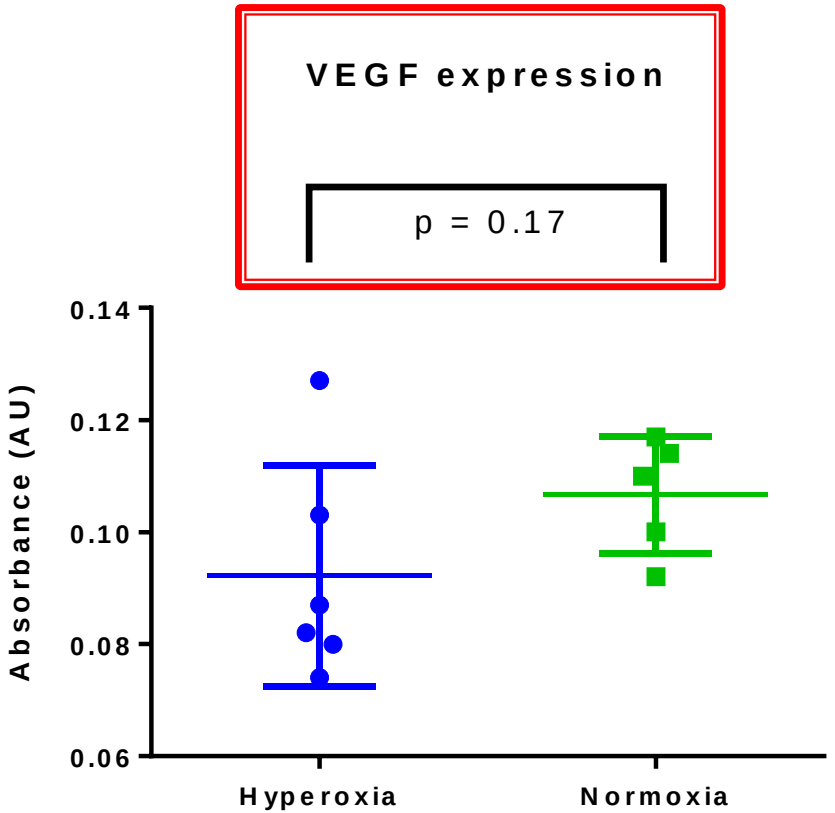
- signal protein
- macrophages
  - Induce fever / Systemic inflammation
  - Apoptosis
  - inhibit tumor growth
- Potent inducer of neo-vascularization

VEGF

- signal protein
- many cells
- stimulates the formation of blood vessels

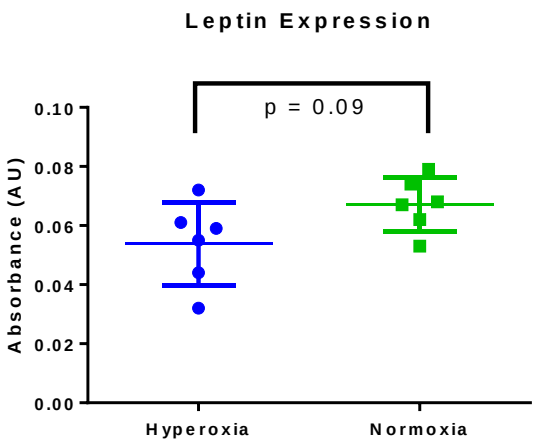
Results – Cytokine Expression

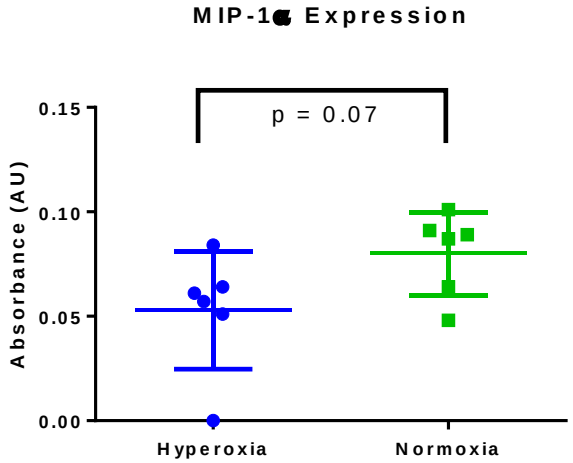




Results – Cytokine Expression

- More trends





Adipokine implicated in the pathogenesis of chronic inflammation

Increased levels linked with risk of
Type 2 diabetes
Cardiovascular disease
Cancer

Macrophage inflammatory protein

– found to be elevated in patients with depression

Anti-inflammatory

Conclusions

- First study to demonstrate
  - at a P_iO_2 320 Torr
  - pro-angiogenic stem cell mobilization
  - suppression of an inflammatory cytokine

- Suggests the possibility of proangiogenic stem cell mobilization in humans at much lower oxygen pressure than previously postulated.

Implications

- Low intensity intermittent oxygen therapy may enhance re-vascularization

- Increases questions regarding shams that intermittently increase $PiO_{2,}$ even at relatively low levels

Future Directions

- Repeat this experiment in humans

Thank you

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Questions?

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